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Published
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(54) Title: USE OF TRANSGLUTAMINASE INHIBITOR FOR THE TREATMENT OF SCAR TISSUE

(57) Abstract

The invention relates to the therapeutic treatment of hypertrophic scar tissue within a composition comprising a non-toxic transglutaminase inhibitor, such as putrescine, or a pharmaceutically acceptable acid addition salt thereof, and a pharmaceutically acceptable carrier or diluent. A successful method for treating hypertrophic scar tissue with such compositions, is also disclosed.

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USE OF TRANSGLUTAMINASE INHIBITOR FOR THE TREATMENT OF SCAR TISSUE

BACKGROUND OF THE INVENTION

The present invention relates to wound therapy, and in particular to the therapeutic treatment of hypertrophic scar tissue.

Scar tissue is formed during healing of wounds, caused for example by burn, traumatic injury and elective operative incisions. Often unpredictably, hypertrophy of the scar tissue occurs. Hypertrophic scar formation is characterized by the accumulation of collagen type III out of proportion to collagen type I.

Current procedures and materials for wound treatment include the use of compounds with potentially serious side effects, to highly invasive excisional procedures.

In accordance with one aspect of the invention, a composition for the therapeutic treatment of hypertrophic scar tissue is provided, comprising a non-toxic transglutaminase inhibitor having a free amino group, or a pharmaceutically acid addition salt thereof, and a pharmaceutically acceptable carrier or diluent.

According to another aspect of the invention, a method of treating hypertrophic scar tissue is also provided, comprising applying to the scar tissue an effective amount of a non-toxic transglutaminase inhibitor having a free amino group or a pharmaceutically acceptable acid addition salt thereof, and a pharmaceutically acceptable carrier or diluent.

Transglutaminases are enzymes present in plasma and various tissues which form isopeptide bonds between reactive glutaminyl groups and the e-amino group of lysine in certain proteins. For example, type III procollagen has been shown to be a specific and avid substrate for transglutaminase. During skin wound healing it appears that type III procollagen amino peptide (PIIP) is cross-linked to other components of the wound matrix, such as fibrin and fibronectin, by tissue transglutaminase. It is therefore hypothesized that if a transglutaminase inhibitor having a free amino group is introduced to the wound site, its free amino group will preferentially bind to the glutaminyl group, and thus inhibit the intended protein substrate from cross-linking, forming an inert analog-amine adduct instead.

Known non-toxic transglutaminase inhibitors of this type include

aminoacetonitrile, (dansyl) cadaverine (1,5-diaminopentane), spermidine and putrescine (1,4-diaminobutane). All of these compounds are non-toxic primary amines.

More specifically, aminoacetonitrile is a primary aliphatic lower-alkyl (C1-5) monoamine. Spermidine is a primary aliphatic aklylamine. Putrescine and (dansyl) cadaverine are primary aliphatic lower-alkyl (C1-5) polyamines. Other similar, non-toxic primary amines of these types are also contemplated.

It will be appreciated by those skilled in the art that the active compounds may be usefully applied in the form of pharmaceutically acceptable acid addition salts such as hydrochlorides and hydrogen sulfates.

The pharmaceutically acceptable carrier is typically a eutectic cream or ointment to facilitate spreading over the wound area. For topical application, mineral oil has been found particularly suitable. Other suitable carriers include polyethylene glycols.

For topical applications, the effective amount of the active compound is in the range of 25 to 100 mM, and preferably about 50 mM.

Once the composition is applied to the wound it may advantageously be occluded with a dressing or incorporated into a transepidermal patch dressing.

It will be appreciated that, although the compositions according to the invention are particularly useful for topical application to external wounds, it is also to be expected to be of value in the treatment of internal scar tissue. In such cases, the composition may be applied by catheter infusion or by an implantable time release mechanism. One specific example is diffusion through the elastomer coating of a breast implant.

Figures 1 to 8 are photographic illustrations of the effects of the compositions according to the invention on hypertrophic scar tissue in human patients.

DESCRIPTION OF THE PREFERRED EMBODIMENT

In the examples which follow, the active compound employed was putrescine.

Putrescine was selected as it is naturally occurring, highly specific and readily available.

Putrescine (Sigma Chemical Co., St. Louis, Mo.) was compounded in a eutectic base (Glaxo Canada Ltd., Toronto, Ont.) at 0.8% (W/V) concentration (50 mM).

Patients applied the ointment daily and occluded the area with Duoderm CGF^R or Actiderm^R (Convatec, Princeton, N.J.). If removed for any reason the cream was to be reapplied as soon as possible. Patients were to report the presence of any reaction which developed.

All photographic documentation was carried out by the professional medical photography department using similar lighting and techniques for each wound at the various times recorded.

CASE 1

A 32-year old male having burns involving the trunk and lower extremities resulting from a motor vehicle accident was treated. Burn management consisted of excision and grafting of the lower extremities and a full take occurred in all areas except over the Achilles tendons bilaterally. The patient was discharged home with contractures of the right leg preventing full extension.

At a three-week follow-up, the patient was unchanged and gross hypertrophy of the right leg and, to a lesser extent, the left leg was apparent. The patient then was treated with the composition of the invention for one month to the right leg only. During this period, ulceration over tendo Achilles healed fully, but that over the left did not show signs of improvement despite treatment with dressings. The scars were less hypertrophic on the right leg.

At a three-month follow-up, the right knee had a full range of movement and signs of hypertrophy on the right had resolved. whereas the left side was still quite red and raised. The scars on the left also felt quite hard, even though pressure garments were

continuously used.

Figures 1 to 4 are photographs of the patient. Figures 1 and 2 are side and posterior views of the patient prior to treatment while Figures 3 and 4 are side and posterior views of the patient post-treatment.

The results obtained in this case exemplify the utility of the compositions of the invention in treating early hypertrophy in burn patients, preventing the need for surgical release of contractures, and allowing stabilization of unhealed areas.

CASE 2

A 3-year old female with a scald burn sustained 9 months previously and treated by excision and grafting was treated. The patient had scar contractures which were fairly mature, had fixed deformities of the toes which prevented normal shoe wear, and was developing minor ulceration from her special footwear.

A composition according to the invention was applied for one month and the scars were seen to soften with improvement in the skin stability over that time period. At a three month follow-up, some residual deformity persisted but the patient had regained full range of motion and was again able to wear normal footwear.

Figures 5 and 6 are photographs of the patient, Figure 5 being taken prior to treatment and Figure 6 post-treatment. These results show the utility of the compositions of the invention in the treatment of mature burn scar tissue.

CASE 3

A 12-year old female was presented one year after an iliac crest free bone flap reconstruction for a dermatofibroma of the mandible resected 6 years previously. The patient had gross hypertrophy of her scars along the entire suture line. In the postauricular area, she had a keloid-like scar which caused protruding of the ear itself.

The scars were excised and the patient was treated with a composition of the

invention for one month. Since the treatment, there has been no recurrence of hypertrophy in the excised areas. Further follow-up at 6 months reveals maturation of scars and quality similar to that seen typically in much older ones.

Figures 7 and 8 are photographs of the patient. Figure 7 was taken pretreatment, while Figure 8 was taken post-treatment. These results show the utility of the compositions of the invention in preventing hypertrophic scar tissue formation.

As seen in the first of three cases studied subsequently, skin stability is not adversely affected by topical putrescine. In fact, epithelialization occurs more rapidly in the presence of the composition of the invention.

The first patient (case 1) was assessed subsequently at two years post injury by the Workers Compensation Board physician who found a thirty percent greater range of motion on the treated right lower extremity as compared to the left. He had no evidence of hypertrophic scar contracture on the right at this time. However, there were obvious contractures on the left, which was initially the less severely injured extremity. This exemplifies the use of the composition of the invention in treating early hypertrophy in burn patients, preventing the need for surgical release of contractures and allowing stabilization of unhealed areas.

The second case (case 2) demonstrates the application to established contractures with reasonable improvement in appearance and function. It also exemplifies the use of the composition under a pressure garment.

The last example (case 3) is that of prophylactic use in a patient prone to hypertrophic scar formation. Overall wound healing was not adversely affected and hypertrophy was well controlled.

In no case did wounds undergoing treatment fail to heal normally aside from varying degrees of hypertrophy. Patients tolerated the composition well. In a previous

uncontrolled study as well as in the present work virtually no side effects were witnessed. Out of a total of one hundred and fifteen patients treated, only one patient developed a rash which necessitated discontinuance of the treatment. He went on to require excision and grafting of his hypertrophic burn scars. No one else required further revision, being satisfied with the clinical improvement at one year post treatment.

A major advantage of the treatment according to the invention is the ease of use. A once daily application under an occlusive dressing is relatively convenient. Further advantages are the lack of associated morbidity seen with other treatment modulities such as the skin atrophy and painful injection from intralesional steroid treatment. Moreover, anaplasia as seen in irradiated scars is unlikely. It is also apparent that the composition may be readily applied beneath pressure garments.

WE CLAIM

1. A therapeutic composition, comprising a non-toxic transglutaminase inhibitor having a free amino group or a pharmaceutically acceptable acid addition salt thereof, and a pharmaceutically acceptable carrier or diluent.

2. A composition according to claim 1, wherein the inhibitor is a primary amine.

3. A composition according to claim 2, wherein the amine is a polyamine.

4. A composition according to claim 3, wherein the amine is aliphatic.

5. A composition according to claim 4, wherein the amine is a C1-5 alkyl amine.

6. A composition according to claim 5, wherein the amine is putrescine.

7. A composition according to claim 6, wherein the carrier is a eutectic cream or ointment.

8. A method of treating hypertrophic scar tissue, comprising applying to the scar tissue an effective amount of a non-toxic transglutaminase inhibitor having a free amino group or a pharmaceutically acceptable acid addition salt thereof, and a pharmaceutically acceptable carrier or diluent.

9. A method according to claim 8, wherein the effective amount is 25 to 100 mM.

10. A method according to claim 9, wherein the effective amount is about 50 mM.

11. A method according to claim 10, wherein the inhibitor is a

primary amine.

12. A method according to claim 11, wherein the amine is a polyamine.

13. A method according to claim 12, wherein the amine is a C1-5 alkyl amine.

14. A method according to claim 13, wherein the amine is putrescine.

15. A method according to claim 14, wherein the carrier is a eutectic cream or ointment.

FIGURE 1

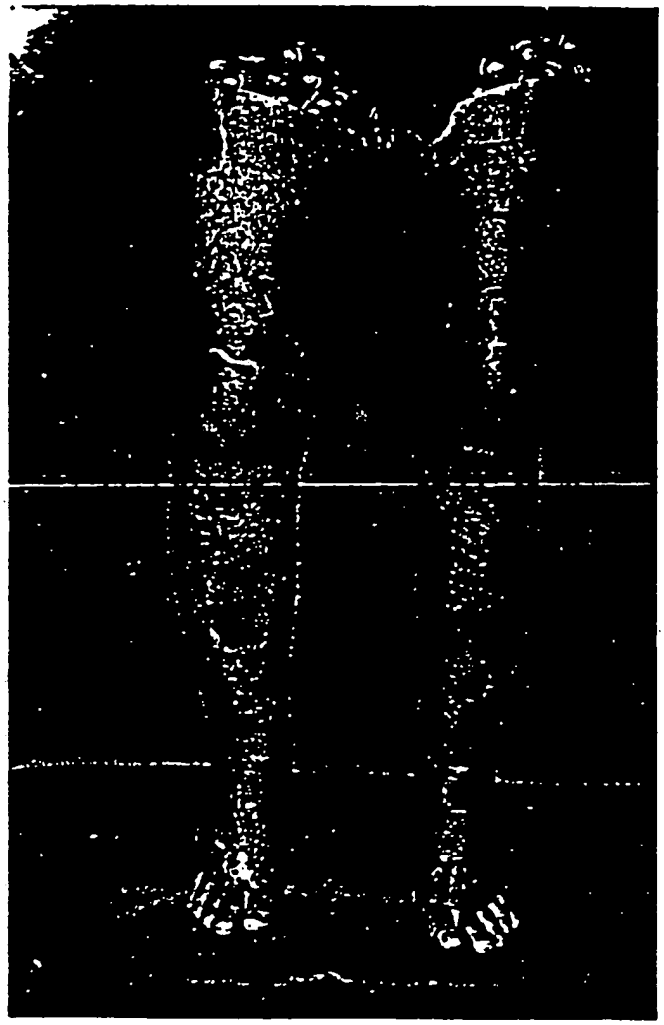


FIGURE 2

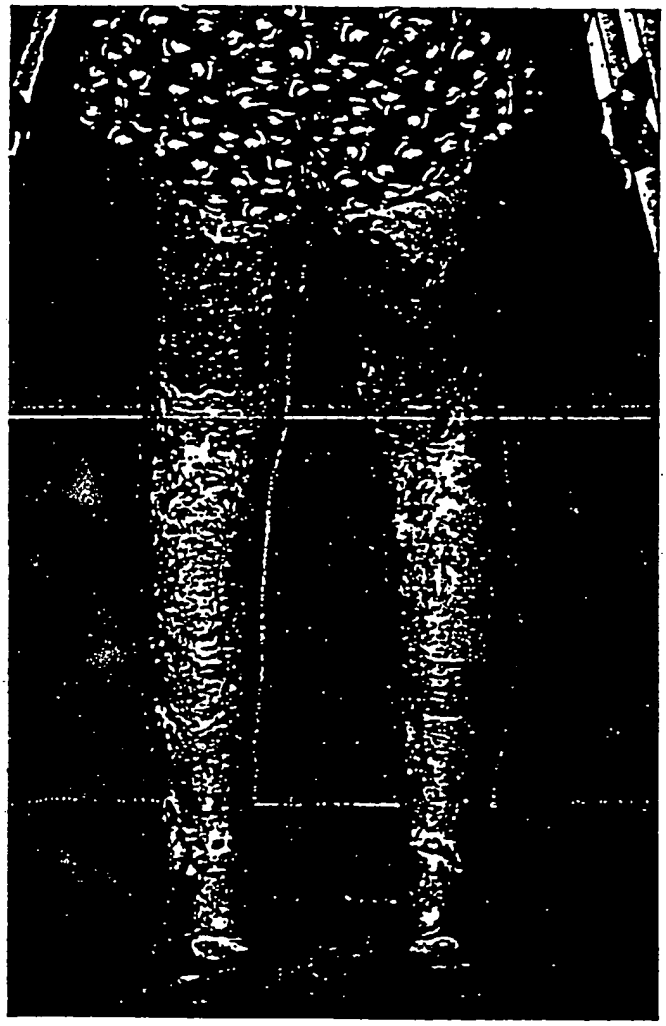


FIGURE 3

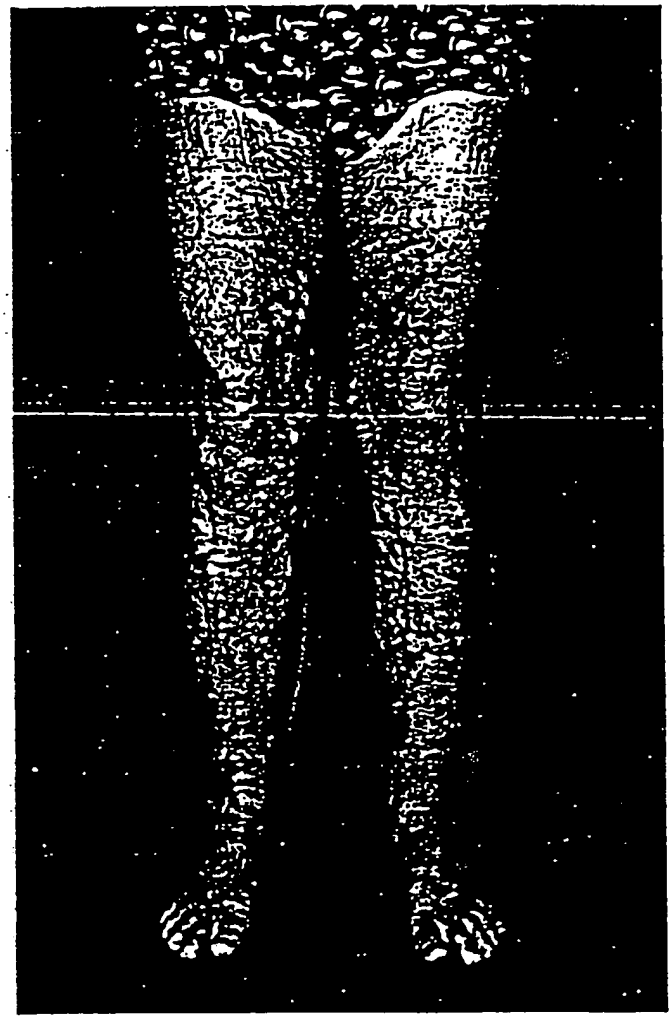


FIGURE 4

FIGURE 5

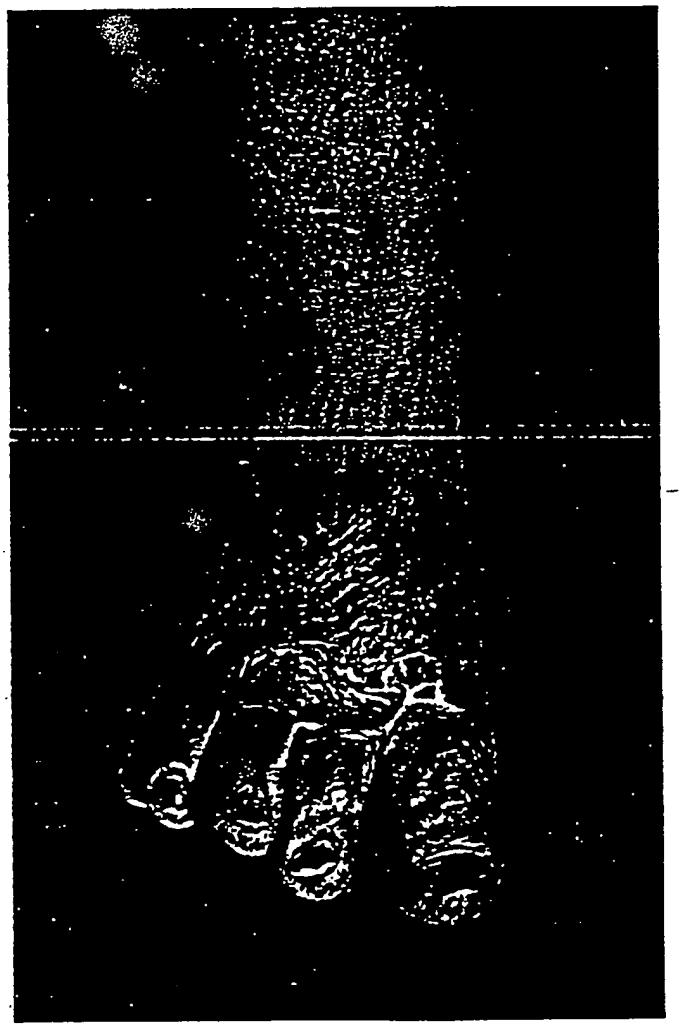


FIGURE 6

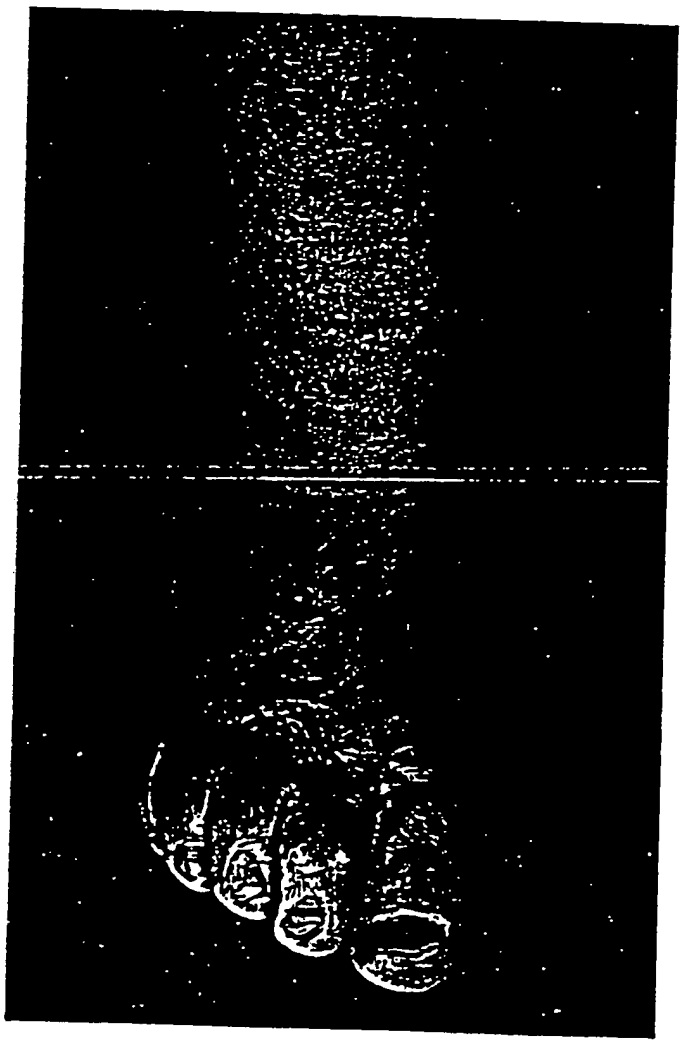


FIGURE 7

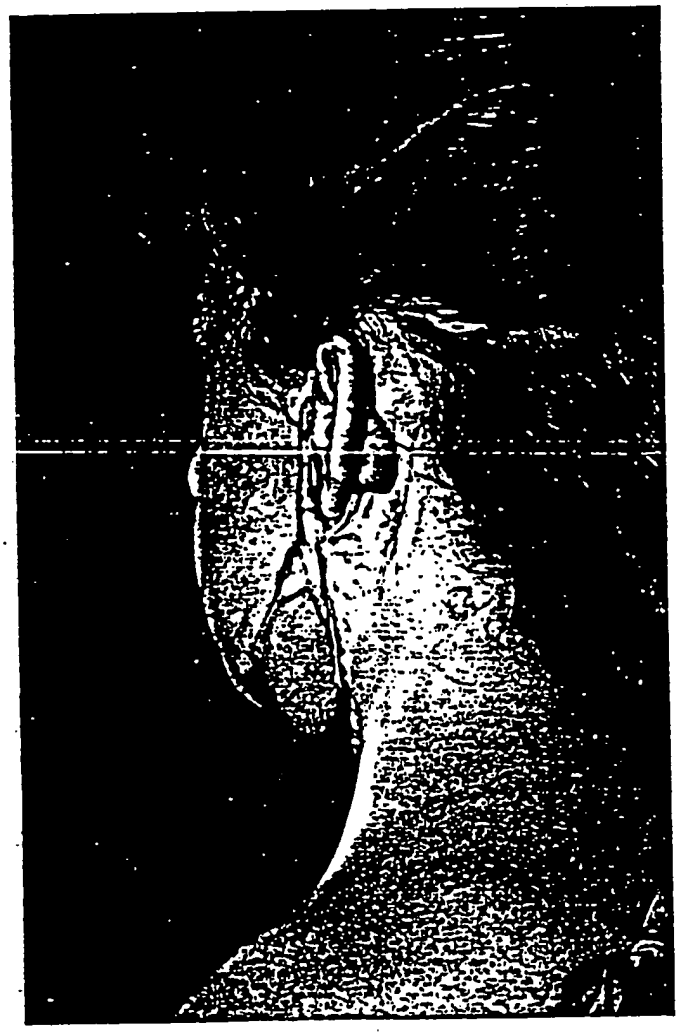


FIGURE 8

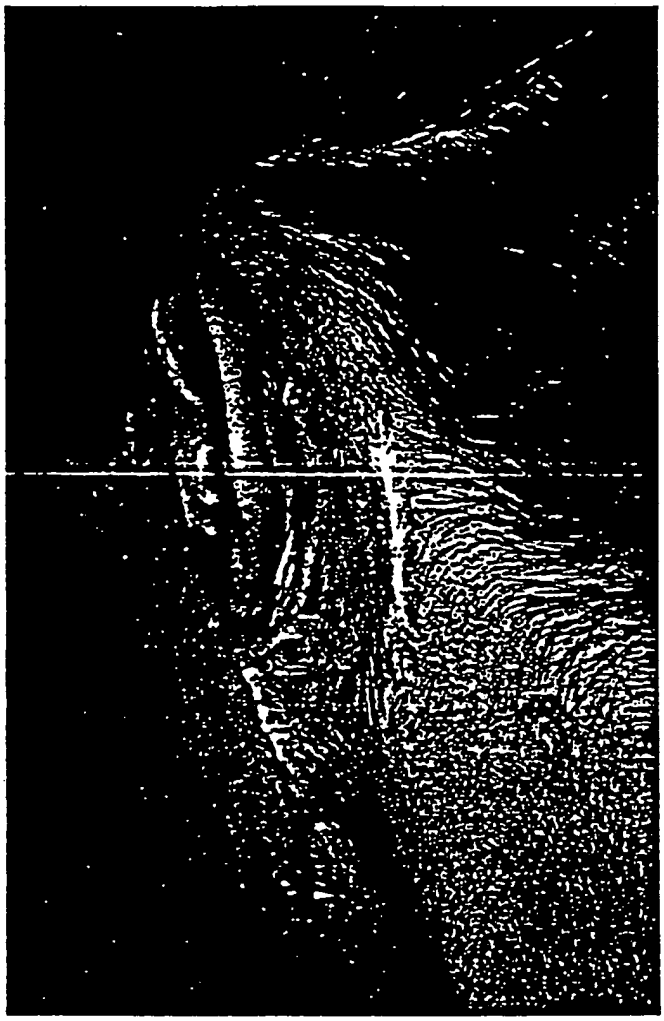
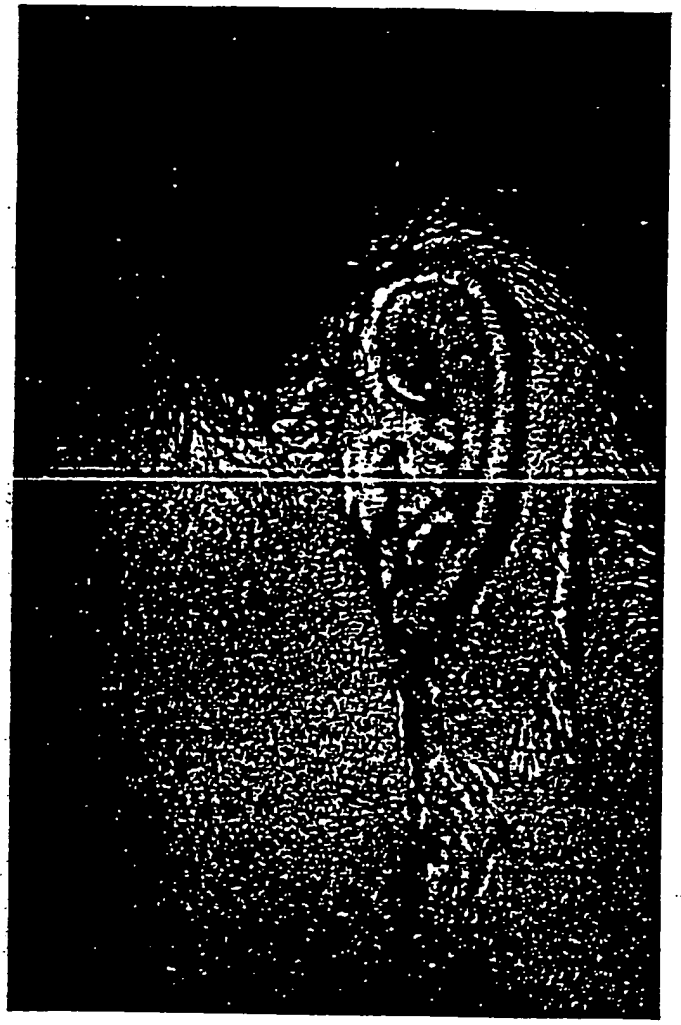


FIGURE 9



International Application No PCT/CA 92/00123

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl.5 A 61 K 31/13 A 61 K 31/135 A 61 K 31/275

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl.5	A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document,[11] with indication, where appropriate, of the relevant passages[12]	Relevant to Claim No.[13]
X	US,A,4485088 (CHVAPIL) 27 November 1984, see abstract	1,2,8-11
X	US,A,4507321 (RAISFELD et al.) 26 March 1985, see abstract; examples; columns 4-9; column 2, line 33 - column 4, line 58	1-7
X	US,A,4997854 (KAGAN) 5 March 1991, see abstract; column 12, line 40 - column 13, line 45; claims 1-4	1-5,8-13
X	WO,A,9110427 (UNIV. OF TEXAS) 25 July 1991, see abstract; page 9, lines 8-18; page 14, lines 18-26	1-6

-/-

° Special categories of cited documents :[10]

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"P" document published prior to the international filing date but later than the priority date claimed

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"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27-05-1992	26 JUN 1992
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MISS T. TAZELAAR

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
X	Annals of Surgery, vol. 193, no. 5, May 1981, E.E. PEACOCK: "Pharmacologic control of surface scarring in human beings", pages 592-597, see abstract	1,2,8-11

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☒ OBSERVATION WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 8-15 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compounds/compositions.

2. ☐ Claim numbers because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. ☐ Claim numbers because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows.

1 ☐ As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims

3 ☐ No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers

4 ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. CA 9200123 SA 57517

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 18/06/92
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Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A- 4485088	27-11-84	None	
US-A- 4507321	26-03-85	None	
US-A- 4997854	05-03-91	None	
WO-A- 9110427	25-07-91	AU-A- 7478991	05-08-91